UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF PATHOGENIC *VIBRIO* SPP AND MOLECULAR CHARACTERISATION OF SELECTED *VIBRIO CHOLERAE* SEROVARS ISOLATED FROM SEAFOOD IN MALAYSIA

NASRELDIN ELHADI HUSSEIN MOHAMED

FSMB 2002 6

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By

NASRELDIN ELHADI HUSSEIN MOHAMED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

February 2002

Dedicated to my parents,
my lovely son,
my brothers
my sisters
and for Sitelbenat for being Sitelbenat

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy.

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Chairman: Associate Professor Son Radu, Ph.D.

Faculty: Food Science and Biotechnology

The global resurgence of cholera is an important public health challenge as the number of countries affected by this infection continues to increase. Cholera continues to be a major health challenge in Malaysia. To adequately control the infection requires a thorough understanding of its modes of transmission and implementing a comprehensive programme which takes into account not only the health aspect but also social, economic, behavioral and political dimension as well. The food-borne cholera and other pathogenic vibrios have not been thoroughly investigated in food in Malaysia. This study demonstrated the presence of toxigenic and non-toxigenic *Vibrio cholerae* O1, *Vibrio cholerae* O139, *Vibrio cholerae* non-O1/O139 and other vibrios pathogenic to mankind, which were isolated from seafood marketed from wet-markets and supermarkets from different locations in Malaysia from July 1998 to June 1999.

In this prevalence study, a total of 768 seafood samples were examined for the presence of pathogenic *Vibrio* species. A total of 431 of 768 (56.1%) seafood samples

were contaminated with Vibrios. The frequencies of *Vibrio* contamination in seafood were: shrimp (*Panaeus indicus*) 58.6%, squids (*Logio sp.*) 44%, squids (*Sepia sp.*) 60%, cockles (*Anadara granosa*) 82%, blue crabs (*Callinectes sapidus*) 62%, Shellfish (*Lithophaga malaccana*) 48% and peel mussels (*Perna viridis*) 24%. *V. damsela* and *V. alginolyticus* was the most frequently isolated species, followed by *V. meschnikovii*, *V. fluvalis*, *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. mimicus*. Of the 433 shrimp samples examined, 35.5% (254/433) were found to be positive for *Vibrio*, which yielded 791 isolates of *Vibrios*. Thus, 68.2 (58/85), 62.2 (28/45), 88 (22/25), 96 (24/25), 25 (5/20) and 100% (48/48) of the samples obtained from Selayang, Kajang, Bangi in Selangor; Seremban in Negri Sembilan; Penang (North Peninsula Malaysia); and Kuching in Sarawak (on Borneo island) yielded 158, 74, 62, 68, 14, and 231 isolates of *Vibrios*, respectively. Examination of 185 shrimp samples from Serdang wet market in Selangor over a nine-month period gave a contamination rate of 37.2% with incidence for the months ranged from 15 to 40%.

The incidence of *V. cholerae* in the various shrimp samples obtained from wet markets and supermarkets showed that a total of 14 (3.2%) and 3 (0.69%) of the 433 samples were contaminated with *V. cholerae* non-O1/O139 and O139, respectively. The highest number of *V. cholerae* non-O1/O139 were found in the shrimp samples collected from Kuching in Sarawak supermarkets with contamination rate of 10.4% (5/48) which yielded 20 isolates, followed by Kajang wet market (5.8% or 5/45) which yielded 16 isolates. The lowest rate of *V. cholerae* non-O1/O139 contamination were observed in Selayang wet market (2.2% or 1/45), Serdang wet market (1% or 2/185)

and Penang wet market (10% or 2/20). *V. cholerae* non-O1/O139 was not detected in shrimp samples collected from Bangi wet market and Seremban supermarkets. *V. cholerae* O139 was isolated only from Kuching location with contamination rate of 4.1% (3/48).

A total of 75 squids (*Logio sp*), 60 squids (*Spia* sp), 50 cockles (*Anadara granosa*), 50 blue crabs (*Callinectes sapidus*), 50 shellfish (*Lithophaga malaccana*), 50 peel mussels (*Perna viridis*) samples, were examined for the presence of pathogenic *Vibrios* at different wet markets and supermarkets in Selangor location [Petaling Jaya, Subang Jaya, Serdang, Seri Kembangan, Seri Petaling, and Kajang] in Malaysia. The contamination rate of *Vibrio* in Squids (*Logio* sp) and Squids (*Spia* sp) samples taken from Petaling Jaya and Subang Jaya supermarkets were 37.5% (15/40), 51.4% (18/35), 46.6% (14/30) and 73.3 (22/30) respectively. The cockles (*Anadara granosa*) collected from Petaling Jaya, Subang Jaya, Serdang and Seri Kembangan had contamination rate of 90% (9/10), 80% (8/10), 86.6% (13/15) and 73.3% (11/15) respectively. The frequencies of *Vibrios* contamination in blue crabs which were sampled from four locations; Seri Kembangan supermarket, Seri Petaling, Seri Kembangan wet market were 50% (5/10), 53.3% (8/15), 40% (2/5), 50% (5/10) respectively with the highest contamination observed in the 20 samples taken from Serdang wet market were all positive with a rate of 80% (16/20).

The prevalence of *V. cholerae* O1, O139 and non-O1/O139 from different types of seafood (squids, cockles, blue crabs, shellfish, peel mussels) sampled from different

location in Petaling Jaya, Subang, Selayang, Seri Petaling, Serdang, Seri Kembangan and Kajang from a total of 335 samples, gives contamination rates of 4.47% (15/335) which yielded 33 isolates of *V. cholerae* non-O1/O139 and 0.59% (2/335) which yielded 3 isolates of *V. cholerae* O139. *V. cholerae* O139 was recovered from cockles (*Anadara granosa*) from Serdang wet market and blue crab obtained from Seri Kembangan wet market, with contamination rate of 6.6% (1/15) which yielded 1 isolate, and 10% (1/10) which yielded 2 isolates, respectively. *V. choleare* O1 serotype Ogawa was recovered from blue crab sample obtained from Serdang wet market with contamination rate of 5% (1/20) which yielded 1 isolate.

In this study growth survival of *V. cholerae* O1 was investigated in soya bean milk, coconut milk, glutinous rice, rice, shrimp and crab at different five storage temperature (4, 10, 15, 25 and 35 ^{O}C). The growth of *V. cholerae* O1 in different five selected food was very slow at 4, 10 and 15 ^{O}C. Growth of *V. choleare* O1 was very significant in all food studied at 25 and 35 ^{O}C. *V. cholerae* O1 inoculated into crabs meat and shrimp entered the growth phase after 3 hours as compare to soya bean milk, rice, glutinous rice and coconut milk show growth phase after 12 hours.

In this study all strains of *V. cholerae* isolated from seafoods were screened for the antibiotic susceptibility and plasmid profiles. *V. cholerae* O1 and O139 isolates do not harbor plasmid, plasmid were detected only in two isolates of *V. cholerae* non-O1/O139 ranging in size from 3.4 to 4.6 megaDalton. The presence or absence of *ctx* and *tcp*A genes in all 97 isolates of *V. cholerae* were examined by using Multiplex PCR and

DNA colony hybridization test. Shrimp collected from Kuching in Sarawak resulting 20 isolates of *V. cholerae* non-O1/O139 and 11 isolates of *V. cholerae* O139. All isolates of *V. cholerae* O1 and O139 were positive for *ctx* and *tcp*A genes and belong to El Tor biotype. 19.5% of *V. cholerae* isolates were positive, and the result obtained by multiplex PCR is completely agreed with the result of DNA colony hybridization. The results obtained by PCR and DNA colony hybridization of positive strains of *V. cholerae* O1, O139 and non-O1/O139 isolated from seafood provide evidence that there is high priority of contamination of finding toxigenic strains of *V. cholerae* in seafood marketed in wet-markets and supermarkets in Malaysia. The results of this study demonstrated that toxigenic *V. cholerae* was detected in shrimp, cockles and crabs marketed in wet-markets and supermarkets and 19.5% of isolates contain genes encoding virulence factors. However, the significance of the presence of such *V. cholerae* O1, O139 and non-O1/O139 strains in seafood products in relation to potential hazards to public health is questionable. Further studies are required to determine the pathogenic potential and also the ecology and taxonomy of this species in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

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Oleh

Nasreldin Hussein Mohamed

February 2002

Pengerusi: **Profesor Madya Dr. Son Radu**

Fakulti: **Sains Makanan dan Bioteknologi**

Kemunculan penyakit kolera secara global adalah cabaran kesihatan yang penting kerana bilangan negara yang dipengaruhi infeksi ini semakin meningkat. Kolera terus menjadi cabaran kesihatan yang utama di Malaysia. Pengertian yang mendalam tentang mod transmisi dan pelaksanaan program komprehensif yang melibatkan aspek kesihatan, social, ekonomi, tabiat dan dimensi politik adalah penting untuk mengawal infeksi ini. Penyakit kolera belum dikaji sepenuhnya di Malaysia. Kajian ini menunjukkan kehadiran *Vibrio cholerae* O1, *Vibrio cholerae* O139, *Vibrio cholerae* non-O1/O139 dan *Vibrio* lain yang patogenik kepada manusia yang dipencilkan daripada makanan laut di pasar malam dan pasaraya dari lokasi yang berbeza di Malaysia dari Julai 1998 hingga Jun 1999.

Di dalam kajian ini, sejumlah 768 sampel makanan laut telah dikaji untuk kehadiran spesis *Vibrio*. Sejumlah 431 daripada 768 (56.1%) sampel makanan laut telah

dikontaminasi dengan *Vibrio*. Frekuensi kontaminasi *Vibrio* di dalam makanan laut adalah: udang (*Panaeus indicus*) 58.6%, sotong (*Logio sp.*) 44%, sotong (*Sepia sp.*) 60%, tiram (*Anadara granosa*) 82%, ketam (*Callinectes sapidus*) 62%, shellfish (*Lithopaga malaccana*) 48% dan peeled mussels (*Perna viridis*) 24%. *Vibrio damsela* dan *V. alginolyticus* adalah sepsis yang paling banyak dipencilkan, diikuti oleh *V. meschnovii, V. fluvialis, V. parahaemolyticus, V. vulnificus, V. cholerae* dan *V. mimicus*. Daripada 433 sampel udang yang dikaji 35.5% (254/433) didapati positif untuk *Vibrio*, ini memberi jumlah sebanyak 791 pencilan *Vibrio*. Oleh kerana itu, 68.2 (58/58), 62.2 (28/45), 88 (22/25), 96 (24/25), 25 (5/20) dan 100% (48/48) sample yang diperolehi dari Selayang, Kajang, Bangi di Selangor; Seremban di Negeri Sembilan; Pulau Pinang (Utara Semenanjung Malaysia) dan Kuching di Sarawak (Kepulauan Borneo) memberi 158, 74, 62, 68, 14 dan 231 pencilan *Vibrio* masing-masing. Ujian ke atas 185 sampel udang darii pasar malam di Selangor selama 9 bulan memberi ladar kontaminasi sebanyak 37.2% di mana insiden untuk bulan-bulan tersebut adalah dalam lingkungan 15 hingga 40%.

Insiden *Vibrio* di malam pelbagai sampel udang yang diperolehi dari pasar malam dan pasaraya-pasaraya adalah sebanyak 14 (3.2%) dan 3 (0.69%) daripada 433 sampel yang dikontaminasi dengan *Vibrio cholerae* non-O139 daan O139, masing-masing. Bilangan tertinggi *Vibrio cholerae* telah dijumpai di dalam sampel udang yang diperolehi dari pasaraya di Kuching, Sarawak dengan kadar kontaminasi sebanyak 10.4% (5/48) dan memberi sebanyak 20 pencilan, diikuti dengan pasar malam Kajang (5.8% atau 5/48) yang memberi sebanyak 16 pencilan. Kadar kontaminasi *Vibrio cholerae* yang terendah diperhatikan di pasar malam Selayang (2.2% atau 1/45), pasar malam Serdang (1% atau

2/185) dan pasar malam Pulau Pinang (10% atau 2/20). *Vibrio cholerae* non-O139 tidak dikesan di dalam sample udang yang diperolehi dari pasaraya Bangi dan pasaraya Seremban. *Vibrio cholerae* O139 hanya dipencilkan dari lokasi Kuching dengan kadar kontaminasi sebanyak 4.1%.

Sejumlah 75 sotong (*Logio sp.*), 60 sotong (*Spia sp.*), 50 tiram (*Anadara granosa*), 50 ketam biru (*Callinectes sapidus*), 50 shellfish (*Lithopaga malaccana*), peeled mussels (*Perna viridis*) sampel telah dikaji untuk kehadiran *Vibrio* di pasar malam dan pasaraya yang berbeza di Selangor (Petaling Jaya, Subang, Serdang, Seri Kembangan, Seri Petaling dan Kajang) di Malaysia kadar kontaminasi *Vibrio* di dalam sotong (*Logio sp.*) dan sotong (*Spia sp.*) sampel diperolehi dari Petaling Jaya dan Subang Jaya adalah 37.5% (15/40), 51.4% (18/35), 46.6% (14/30) dan 73.3 (22/30) masing-masing. Tiram (*Anadara granosa*) yang dikumpul dari Petaling Jaya, Subang Jaya, Serdang dan Seri Kembangan mengalami kadar kontaminasi sebanyak 90% (9/10), 80% (8/10), 86.6% (13/15) dan 73.3% (11/15) masing-masing. Frekuensi kadar kontaminasi di dalam ketam biru yang diambil dari 4 lokasi iaitu pasaraya Seri Kembangan, Seri Petaling, pasar malam Seri Kembangan adalah 50%, 53.3%, 40%, 50% masing-masing dengan kadar kontaminasi tertinggi diperhatikan di dalam 20 sampel yang diambil dari pasar malam Serdang di mana kadarnya adalah 40%.

Taburan *Vibrio* cholerae non-O139 dan O139 dari pelbagai sampel makanan laut yang diperolehi dari lokasi yang berbeza di Petaling Jaya, Subang, Selayang, Sri Petaling, Serdang, Seri Kembangan dan Kajang merangkumi sebanyak 335 sampel dan memberi

kadar kontaminasi sebanyak 4.47% dimana 33 pencilan adalah *Vibrio* cholerae non-O139 dan 0.59% memberi 3 pencilan *Vibrio cholerae* O139. *Vibrio cholerae* O139 diperolehi dari tiram di pasar malam Serdang dan ketam biru diperolehi dari pasar malam Seri Kembangan dengan kadar kontaminasi 6.6% dan memberi satu pencilan dan 10% memberi 2 pencilan, masing-masing. *V. cholerae O1 serotype Ogawa* diperolehi daripada sampel ketam biru yang diperolehi dari pasar malam Serdang mengalami kadar kontaminasi 5% dan memberi 1 pencilan.

Kadar pertumbulan *V. cholerae* O1 telah dikaji di dalam susu kacang soya, susu kelapa, nasi kanji, nasi, udang dan ketam pada lima suhu simpanan yang berbeza. Pertumbuhan *V. cholerae* O1 dalam kelima –lima sampel makanan yang berbeza adalah sangat perlahan pada suhu 4, 10 dan 15 C. Pertumbuhan *V. cholerae* O1 adalah amat ketara di dalam kesemua sampel makanan yang dikaji pada suhu 25 dan 35 C. *V. cholerae* O1 diinokulasi di dalam daging ketam dan udang memasuki fasa pertumbuhan selepas 3 jam berbanding dengan susu soya, nasi kanji dan susu kelapa yang menunjukkan fasa pertumbuhan selepas 12 jam.

Menurut kajian ini semua *V. cholerae* yang dipencilkan dari makanan laut telah diskrin untuk kerintangan antibiotik dan profil plasmid. Pencilan *V. cholerae* O1 dan O139 tidak mengandungi plasmid, plasmid hanya dikesan di dalam 2 pencilan *V. cholerae* O1 yang bersaiz 3.4 hingga 4.6 megadalton. Kehadiran atau ketidak hadiran gen CTX dan *Tcp*A di dalam kesemua 97 pencilan *V. cholerae* yang dikaji menggunkan multiplex PCR dan ujian hibridasi koloni DNA. Udang yang dikumpul dari Kuching

Sarawak menghasilkan 20 pencilan *V. cholerae* non-O1/O139 dan 11 pencilan *V. cholerae* O139. Kesemua pencilan *V. cholerae* nonO1/O139 adalah positif dan keputusan ini diperolehi menggunakan multiplex PCR yang selari dengan keputusan ujian hibridasi koloni DNA. Keputusan yang diperolehi dari PCR dan hibridasi koloni DNA adalah positif untuk *V. cholerae* O1, O139 dan non-O1/O139 yang dipencilkan daripada makanan laut serta membuktikan bahawa adanya kontaminasi pencilan *V. cholerae* yang tinggi di dalam makanan laut yang dijual di pasar malam di dalam udang, tiram dan ketam yang dijual di pasar malam dan pasaraya dan 19.5% pencilan mengandungi gen faktor virulen. Walau bagaimanapun kehadiran signifikan *V. cholerae* O1, O139 dan non O1/O139 di dalam produk makanan laut yang berkaitan dengan potensi bahaya kepada kesihatan umum adalah satu persoalan. Kajian yang lebih mendalam perlu dilakukan untuk memastikan kesan patogenik, ekologi dan taksonomi spesis ini di Malaysia.

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NASRELDIN ELHADI HUSSEIN

I certify that an Examination Committee met on 21st February, 2002 to conduct the final examination of Nasreldin Elhadi Hussein Mohamed on his Doctor of Philosophy thesis entitled "Prevalence of Pathogenic *Vibrio* spp and Molecular Characterisation of Selected *Vibrio cholerae* serovars Isolated from Seafood in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 20 MAR 2002

The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for degree of Doctor of Philosophy.

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Date: 13 JUN 2002

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

NASRELDIN ELHADI HUSSEIN MOHAMED

Date: 19-3-2002

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